K043576

AmpliChip CYP450 Test for CYP2C19
510(k) Summary

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1. DEVICE NAME

Trade or Proprietary Name: AmpliChip CYP450 Test

Common or Usual Name: Cytochrome P450 CYP2C19 Test

Classification Name: 21 CRF 862.3360 – Drug Metabolizing Enzyme Genotyping System

2. IDENTIFICATION OF THE LEGALLY MARKETED DEVICE TO WHICH IS CLAIMED EQUIVALENCE

The AmpliChip CYP450 Test for CYP2C19 is equivalent to the AmpliChip CYP450 Test for CYP2D6 (510(k) Premarket Notification K042259, submitted August 20, 2004).

3. DESCRIPTION OF THE DEVICE

The AmpliChip CYP450 Test is based on five major processes: PCR amplification of purified DNA; fragmentation and labeling of the amplified products; hybridization of the amplified products to a microarray and staining of the bound products; scanning of the microarray; and determination of the CYP450 genotype and predicted phenotype.

The AmpliChip CYP450 Test is designed to identify specific nucleic acid sequences and query for the presence of known sequence polymorphisms through analysis of the pattern of hybridization to a series of probes that are specifically complementary either to wild-type or mutant sequences. Microarrays of oligonucleotide probes synthesized on a glass substrate are utilized for the analysis.

4. INTENDED USE OF THE DEVICE

The AmpliChip CYP450 test is intended to identify a patient's CYP2C19 genotype from genomic DNA extracted from a whole blood sample. Information about CYP2C19 genotype may be used as an aid to clinicians in determining therapeutic strategy and treatment dose for therapeutics that are metabolized by the CYP2C19 gene product.

5. SUMMARY OF THE TECHNOLOGICAL CHARACTERISTICS OF THE DEVICE COMPARED TO THE PREDICATE DEVICE

The technological characteristics of the AmpliChip CYP450 Test for CYP2C19 are identical to those of the AmpliChip CYP450 Test for CYP2D6 (K042259).

6. PERFORMANCE DATA FROM THE NON-CLINICAL STUDIES

6.1. Limit of Detection

The limit of detection of the AmpliChip CYP450 Test was determined by analysis of dilutions of two genomic DNA samples to 0.1, 1 and 2 ng DNA/mL. The concentration of the DNA samples was determined by use of a PicoGreen double stranded DNA Quantitation kit. The DNA samples were *2/*2 and *1/*1 for the CYP2C19 gene. The % positivity rate was determined from the number of correct genotype calls. The lowest level of genomic DNA at which a ≥ 95% positivity rate was obtained for correct detection of the CYP2C19 gene was 25 and 2.5 ng.

Table 1: Limit of Detection for the CYPC19 Gene

DNA Amount (ng)	Number of Arrays	Number of Correct Calls	Positivity Rate	95% Confidence Limit
50	144	144	100%	97.5 – 100%
25	144	144	100%	97.5 – 100%
2.5	144	134	93.1%	87.6 – 96.6%

6.2. Specificity

Specificity of the AmpliChip CYP450 Test was evaluated using genomic DNA samples at approximately 2 ng/mL. Results were recorded as correct genotype calls, no calls, or miscalls. Established methods including allele-specific PCR, PCR-RFLP, and DNA sequence analysis were used to establish the reference CYPC19 genotype for the samples.

Two hundred seventy (270) samples containing one CYP2C19 allele with normal predicted enzymatic activity (*1 allele) were tested.

The specificity of the AmpliChip CYP450 Test was calculated by determining the percentage of tested wild-type samples with the correct wild-type genotype identified, as compared to the total number of wild-type samples tested. The specificity of the AmpliChip CYP450 Test for detection of wild-type samples was 100% for the CYP2C19 gene.

Table 2: Specificity for the CYPC19 Gene

CYP2C196 Genotype	Number of Samples Tested	Number of Correct Calls	Number of Miscalls
*1/*1	270	270	0

6.3. Genotype Detection

Method comparison studies were performed using bi-directional DNA sequencing as the comparator for the AmpliChip CYP450 test. DNA sequence analysis for genotype confirmation was performed for 123 samples with CYP2C19 genotype that had been previously analyzed by the AmpliChip CYP450 Test. One sample that was identified as CYP2C19 *2/*2 genotype by RFLP and by the AmpliChip CYP450 Test was shown by sequencing to be a CYP2C19 *2/*10 genotype. With this single miscall, the agreement between the AmpliChip CYP450 Test and sequencing for CYP2C19 alleles was 99.6%.

The results for each allele analyzed in this manner are presented below in Table 3 for CYP2C19 alleles.

Table 3: Sequencing Concordance for CYP2C19 Alleles

CYP2C19 Allele	Number of Alleles Sequenced	AmpliChip Results			
		Correct Calls	Miscalls	No Calls	Percent Agreement
*1	153	153	0	0	100.0%
*2	79	78	1[1]	0	98.7%
*3	14	14	0	0	100.0%
Total	246	245	0	0	99.6%

[1]*One sample, identified as CYP2C19 *2/*2 by RFLP and the AmpliChip CYP450 Test, was shown by sequencing to be a CYP2C19 *2/*10* genotype.*

Genotype detection was evaluated using genomic DNA samples at approximately 50 ng/PCR. In addition to the sequencing confirmation presented above, additional samples were evaluated by established methods including allele-specific PCR and PCR-RFLP in order to determine the reference CYP2C19 genotype for the samples. The percent agreement for genotype detection of the AmpliChip CYP450 Test was calculated by determining the percentage of tested samples with the correct genotype assigned as compared to the total number of samples tested of that genotype.

Genotype detection results for CYP2C19 were calculated for the individual alleles (Table 4) and by sample (Table 5). The overall genotype call rate and percent agreement for CYP2C19 were both 99.7% for all 399 tested samples.

Table 4: Detection of CYP2C19 Alleles

CYP2C19 Allele	Number of Unique Alleles Tested	Number of Correct Calls	Number of Miscalls	Number of No Calls	Percent Agreement	Number of Replicates
*1	647	647	0	0	100%	842
*2	137	136	1	0	99.3%	176
*3	14	14	0	0	100%	32
Total	796	796	0	0	99.9%	1050

Table 5: Detection of Samples by CYP2C19 Genotype

CYP2C19 Genotype	Total Unique Samples	Number of Correct calls	Number of Miscalls	Number of No Calls	Percent Agreement	Genotype Call Rate
*1/*1	270	270	0	0	100.0%	100.0%
*1/*2	101	101	0	0	100.0%	100.0%
*1/*3	6	6	0	0	100.0%	100.0%
*2/*2	15	14	1[1]	0	93.3%	93.3%
*2/*3	6	6	0	0	100.0%	100.0%
*3/*3	1	1	0	0	100.0%	100.0%
Total	399	398	1	0	99.7%	99.7%

[1] *One sample, shown by sequencing to be CYP2C19 *2/*10*, was miscalled as a CYP2C19 *2/*2 genotype.*

6.3.1. Performance Compared to Other CYP450 Genotyping Methods

The sensitivity and specificity of the identification of the CYP2C19 alleles was determined as compared to PCR-RFLP and DNA sequencing. The results are summarized in Table 6.

Table 6: Reference Method for CYP2C19 Allele Identification

Method(s)	Number of Samples Tested	Number of Correct Calls	Number of Miscalls	Number of No Calls
PCR-RFLP	276	276	0	0
DNA Sequencing and PCR-RFLP	123	122	1	0

6.4. Whole System Failure

The robustness of the AmpliChip CYP450 Test was evaluated by testing 100 replicates of genomic DNA purified from a whole blood specimen using the QIAamp DNA Blood kit. The test solution contained approximately 50 ng DNA/PCR of *1/*1 CYP2C19 genotype. There was one System Failure event where no result was obtained due to inability to scan the stained AmpliChip CYP450 Microarray resulting in a Whole System

Failure rate of 1% with a 95% confidence interval from 94.55 - 99.97% due to the instrument or the AmpliChip CYP450 Microarray. There was a 0% Whole System Failure rate due to the AmpliChip CYP450 Test amplification and detection reagents.

Of the 100 valid replicates, one chip failed to scan the initial and subsequent attempts resulting in failure.

6.5. Cross Contamination

Potential carryover contamination was assessed with five runs of alternating two specimens of distinct genotype, along with the appropriate controls. The position of the specimens plus controls was varied between the runs. No carryover contamination was observed; the appropriate CYP2C19 genotype was obtained for all specimens.

6.6. Reproducibility

To evaluate the reproducibility of the AmpliChip CYP450 Test a six-member panel was constructed from cell lines that represent all known 2C19 alleles. The Reproducibility Panel samples were tested at a concentration of 50 ng DNA/PCR. The genotype and predicted phenotype (extensive metabolizer) of the Reproducibility Panel samples are provided in Table 7.

Table 7: AmpliChip CYP450 Test Panel

CYP2C19 Genotype	Predicted Phenotype
*1 / *1	Extensive
*1 / *2	Extensive
*1 / *3	Extensive
*1 / *1	Extensive
*1 / *2	Extensive
*1 / *2	Extensive

Testing was conducted at three sites; including two external sites and a laboratory at Roche Molecular Systems. The Reproducibility Panel was tested in triplicate for five runs by one operator at each of the three sites, using three lots of reagents.

The 809 results from this study are summarized in Table 8. Genotype calls for CYP2C19 were made for 807/809 (99.8%) samples. Two results did not provide a genotype call. There was one incorrect call for CYP2C19 *1/*1 (99.9% [806/807] correct).

Table 8: Reproducibility Panel Results for CYPC19 Genotype

CYP2C19 genotype	Predicted Phenotype	No. Tested	Genotype Calls N (%)	Correct Genotype Calls	Correct Call Rate Estimate (95% CI)
*1 / *1	Extensive	134	134 (100.0)	133	0.99 (0.97)
*1 / *2	Extensive	135	135 (100.0)	135	1.00 (0.98)
*1 / *3	Extensive	135	135 (100.0)	135	1.00 (0.98)
*1 / *1	Extensive	135	135 (100.0)	135	1.00 (0.98)
*1 / *2	Extensive	135	134 (99.3)	134	1.00 (0.98)
*1 / *2	Extensive	135	134 (99.3))	134	1.00 (0.98)
Total		809	807 (99.8)	806	1.00 (0.99)

6.7. Interference Studies

Ten random specimens of whole blood collected in EDTA, adjusted to a level approximating or exceeding 50 ng DNA/PCR were tested in the absence of, and with, elevated levels of one of three potential endogenous interfering substances (albumin, bilirubin or lipids). The endogenous interfering substances were spiked into the specimens from concentrated stock solutions to at high-test levels as defined by the Clinical and Laboratory Standards Institute. These test levels were intended to reproduce the states of hyperalbuminemia, icterus and lipemia in native specimens. Results from this study demonstrated that elevated levels of lipids, bilirubin and albumin in specimens do not to interfere with the performance of the AmpliChip CYP450 Test.

6.8. Conclusions

The AmpliChip CYP450 Test accurately identifies the CYP2C19 genotype of clinical specimens using DNA purified from human blood.

7. CLINICAL VALIDITY

Individual differences in metabolic rates alter the expected relationship between the dose of a drug and its concentration in the blood or the length of time it stays in the blood. Therefore, a polymorphism in the CYP2C19 enzymes can lead to excessive or prolonged therapeutic effect or drug-related toxicity after administration of a "typical" dose by failing to clear a drug from the blood or by changing the pattern of metabolism to produce toxic metabolites. This is particularly true of drugs with a narrow therapeutic index. Adjustment of drug dosage could be beneficial based upon knowledge of these differences in metabolism, particularly for individuals possessing the poor metabolizer phenotype. Table 9 lists some clinically relevant drugs that are known substrates of CYP2C19 enzymes.

Table 9: Clinically Relevant Drug Substrates for Metabolism by CYP2C19 Enzymes

Proton Pump Inhibitors	Anti-epileptics	Others
Omeprazole	Diazepam	Amitriptyline
Lansoprazole	Phenytoin	Clomipramine
Pantoprazole	Phenobarbitone	Cyclophosphamide
		Progesterone

7.1. Geographic Distribution of Allele Frequencies

The vast majority of poor CYP2C19 metabolizers are accounted for by the two common CYP2C19*2 and CYP2C19*3 alleles. Each of these null alleles is caused by a single nucleotide polymorphism that either causes a splice site defect or a stop codon. These two alleles are quite common among Asian populations where approximately 13-23% exhibit the poor metabolizer phenotype. The CYP2C19 poor metabolizer phenotype is present in about 3-5% of Caucasian and African-American populations.

Table 10: Geographic Differences in CYP2C19 Allelic Frequencies

Allele	Predicted Enzymatic Activity	Chinese	Black	Caucasian
*1	Normal			
*2	None	30%	17%	15%
*3	None	5%	<1%	<1%

DEPARTMENT OF HEALTH & HUMAN SERVICES

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JAN 10 2005

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Re: k043576
Trade/Device Name: AmpliChip CYP450 Test
Regulation Number: 21 CFR 862.3360
Regulation Name: Drug Metabolizing Enzyme Genotyping
Regulatory Class: Class II
Product Code: NTI
Dated: December 23, 2004
Received: December 27, 2004

Dear Dr. Kelly:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820).

This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific information about the application of labeling requirements to your device, or questions on the promotion and advertising of your device, please contact the Office of *In Vitro* Diagnostic Device Evaluation and Safety at (240)276-0484. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 443-6597 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html

Sincerely yours,

Cornelia B. Rooks

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Acting Director
Division of Chemistry and Toxicology
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and
Radiological Health

Enclosure

Statement of Indications for Use

510(k) Number (if known): K043576

Device Name: AmpliChip CYP450 Test

Indications for Use

The Roche AmpliChip CYP450 test is intended to identify a patient's CYP2C19 genotype from genomic DNA extracted from a whole blood sample. Information about CYP2C19 genotype may be used as an aid to clinicians in determining therapeutic strategy and treatment dose for therapeutics that are metabolized by the CYP2C19 gene product.

Prescription Use	X	AND/OR	Over-The-Counter Use	
(Per 21 CFR 801 Subpart D)			(21 CFR 801 Subpart C)	

PLEASE DO NOT WRITE BELOW THIS LINE — CONTINUE ON ANOTHER PAGE IF NEEDED

Concurrence of CDRH, Office of Device Evaluation (ODE)

Division Sign-Off

acting

Office of In Vitro Diagnostic Device Evaluation and Safety

510(k) K043576